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The Parental Origin of the Single X Chromosome in Turner Syndrome: Lack of Correlation with Parental Age or Clinical Phenotype

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Summary

We have used X- and Y-linked RFLPs to determine the origin of the single X chromosome in 25 live-born individuals with Turner syndrome. We determined that 18 individuals retained a maternal X (X^m) and that seven retained the paternal X (X^p). No occult mosaicism was detected. We found no differences in either maternal or paternal ages for the two groups. The ratio of maternal X to paternal X is just over 2:1, which is consistent with the expected proportion of meiotic or mitotic products, with equal loss at each step, given the nonviability of 45,Y. Six phenotypic or physiologic characteristics were assessed: (1) birth weight, (2) height percentile at time of testing, (3) presence of a webbed neck, (4) cardiovascular abnormalities, (5) renal abnormalities, and (6) thyroid autoimmunity. There were no significant differences in birth weights or heights between the girls who retained the maternal X or the paternal X. In addition, no differences between the groups could be appreciated in the incidence of the physical, anatomic, or physiologic parameters assessed.

Introduction

The incidence of Turner syndrome, diagnosed following clinical ascertainment, is estimated to be at least 22.2/100,000 females, with more than 50% having an apparent 45,X karyotype. However, the 45,X karyotype is far more common at conception than it is among live borns, since more than 99% of affected conceptuses undergo spontaneous abortion (Hook and Warburton 1983). Neither the mechanisms responsible for the loss of the X chromosome nor the factors which predispose to fetal survival (except for apparent mosaicism) are known. Early studies on the parental origin of the retained X chromosome were performed using Xg-blood-group linkage but were often not informative. Subsequently, the introduction of molecular techniques using specific X-linked RFLPs have made it possible to assign parental origin of the single X chromosome in almost all affected individuals studied. However, current data may be biased by studies on abortuses (Hassold et al. 1985, 1988) and by small numbers of live borns (Connor and Loughlin 1989). In the present study we have examined a large number of live-born individuals with Turner syndrome to determine the parental origin of the retained X chromosome and to determine whether there is a relationship between parental origin and parental age or patient phenotype.

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Material and Methods

Clinical Assessment

Over the past 3 years all 45,X individuals attending the UCLA pediatric endocrine clinic were asked to participate, with the only selection bias being that both parents be available for study. We were able to study

25 individuals and their families, and studies of all these families were informative. The diagnosis of 45,X Turner syndrome had been based on cytogenetic assessment of 25 or more peripheral leukocyte karyotypes by using Giemsa banding. Studies were carried out under an approved institutional Review Board protocol and after obtaining parental consent and patient consent or assent. Parental age was calculated to the nearest month, on the basis of the date of the patient's birth. Birth weights were obtained from the data first appearing in the medical record. Six phenotypic, anatomic, and physiologic findings were assessed: (1) birth weight of the child, (2) latest height percentile as calculated from the Turner syndrome standards adapted from Lyon and Preece (1985), (3) the presence of a nuchal hygroma, loose neck skin, or an actual webbed neck, (4) cardiovascular anomalies, as determined by echocardiography, (5) renal anomalies, as determined by ultrasonography, and (6) presence of thyroid autoimmunity, as determined by antithyroid antibody determinations according to a method described elsewhere (Riley et al. 1981). The age range of patients was 5–24 years at the time of study.

Laboratory Studies

Genomic DNA was obtained from peripheral leukocytes; methods of DNA isolation, restriction digestion, agarose-gel electrophoresis, Southern transfer blotting, ^{32}P labeling by nick-translation, hybridization, and autoradiography were performed, with minor modifications, according to standard procedures (Rigby et al. 1977; Maniatis et al. 1982). Three DNA hybridization probes, all informative with *Taq*I digests, were used: 52A (DXS51), pDP34 (DXYS1), and CRI-S232 (DXS278). CRI-S232 and pDP34 detect invariant Y-specific fragments in addition to X-linked polymorphisms; therefore their use provides a means to assess Y mosaicism. Additional information on the probes and the associated polymorphisms is provided in the legend to figure 1 and is also available in the "DNA committee" report by Kidd et al. (1989).

Results

Eighteen (72%) of the 25 patients retained the maternal X (X^m), and seven (28%) retained the paternal X (X^p). The parental origin of the X chromosome as related to parental age is summarized in table 1, and two examples of parental origin determinations are provided in figure 1. Under the conditions employed in these studies, unsuspected mosaicism for the Y

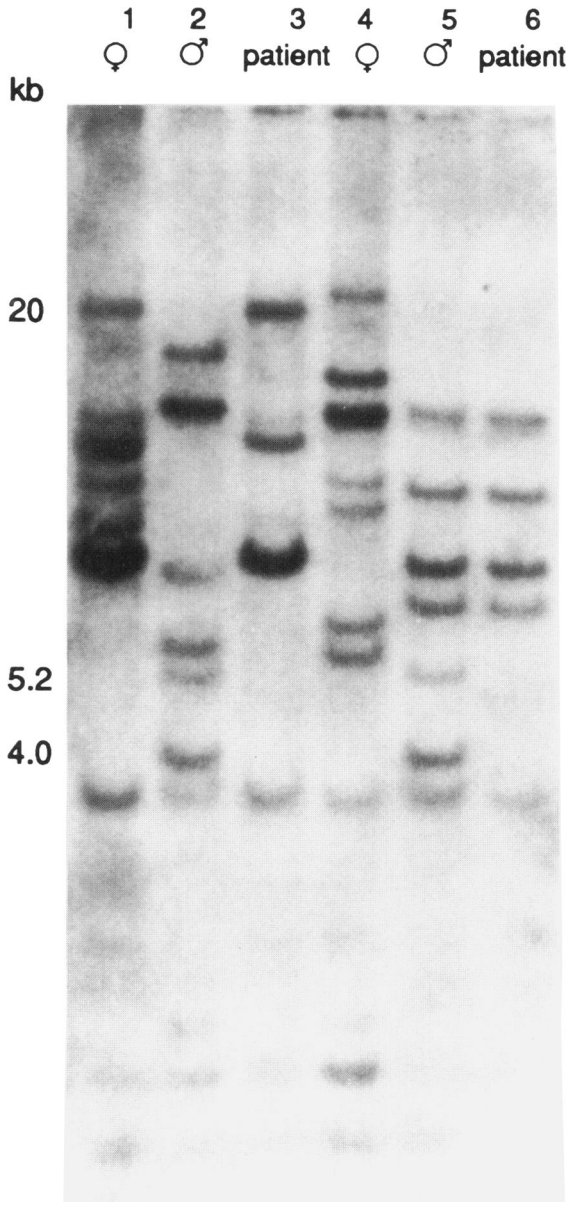

Figure 1 Analysis, in two families, of parental origin of X chromosome, by using *Taq*I-digested DNA probed with CRI-S232. In these two families, the useful polymorphic bands range from just over 20 kb to less than 4.0 kb, with several smaller (faintly seen) fragments of lesser molecular weight which appear to be constant in all individuals. The Y-specific bands at 5.2 and 4.0 kb are seen in lanes 2 and 5. The 45,X patient in lane 3 retains a maternal X, as shown by the fact that she has all her bands in common with her mother (lane 1) but few in common with her father (lane 2). Conversely, the 45,X patient in lane 6 retains her father's X (lane 5), sharing all but the two Y bands and having several fragments not seen in her mother (lane 4).

Table 1

Relationship between Parental Age and Origin of Retained Single X Chromosome

Retained Chromosome	Parental Age (*N*) (years)	
	Maternal[a]	Paternal[a]
X^m	28.5 ± 5.9 (18)	30.1 ± 5.7 (18)
X^p	29.2 ± 7.3 (7)	32.7 ± 7.2 (7)

[a] Difference between age for X^m and that for X^p was not significant.

chromosome was not detected in any of the 25 patients studied. There is no statistical difference between the ages of the mothers of the X^m girls (28.5 ± 5.9 years) as compared with those of the mothers of the X^p girls (29.2 ± 7.3 years). Similarly, there are no statistical differences in the ages of the fathers of the X^m girls (30.1 ± 5.7 years) compared with those of the fathers of the X^p girls (32.7 ± 7.2 years).

The clinical and phenotypic findings in the 25 girls, categorized according to parental origin of the X, are shown in table 2. There were no statistical differences in the birth weights or current height percentiles of the girls in either group. There were a total of 10 cardiac anomalies, primarily of the aortic valve; seven were in girls in the X^m group (aortic stenosis in four, bicuspid aortic valve in two and VSD in one), and three were in girls in the X^p group (aortic stenosis in one and bicuspid aortic valve in two). These were not statistically significant. While there appears to be an increased frequency of webbed neck and renal anomalies (bilateral duplicated collecting system in one, bilateral ureterovesicular obstruction in one, and marked bilateral renal rotation in one) among the X^m girls, the small number of subjects fails to make these significant by either χ^2 analysis or Fisher-exact-test analysis. Thyroid autoantibodies were present in six girls; five were in the X^m and one was in the X^p group. Although the overall prevalence was high, this difference between the groups was not statistically significant ($P > .05$).

Discussion

These data confirm that the parental origin of the single X chromosome in Turner syndrome can be determined by using molecular techniques. In this series of live-born X-monosomic individuals, the proportion of maternal retained X chromosomes, 72%, is similar both to the 75% frequency estimated by Xg-blood-group analysis (Sanger et al. 1977) and to the 71% figure reported by Hassold et al. (1988) for monosomic abortuses. While our data appear to differ from a recent report by Connor and Loughlin (1989), who used molecular techniques similar to ours and found 10 of 10 45,X individuals with a maternal X chromosome, final calculation of the ratio will depend on group analysis of pooled data from numerous sources.

In contrast to the case of 47,XXX aneuploidy, for example, where these techniques can be used to not only assign the origin of the extra X but also to determine whether the event occurred in meiosis I or meiosis II (May et al. 1990), when monosomy exists it is impossible to determine whether the chromosomal loss occurred as a result of a meiotic error during gametogenesis or nondisjunction or anaphase lag following

Table 2

Clinical and Phenotypic Features in 25 Patients with 45,X Turner Syndrome

Retained Chromosome	Clinical/Phenotypic Feature					
	Birth weight (kg)	Turner Height Percentile	Frequency of			
			Webbed Neck	Cardiac Abnormality	Renal Abnormality	Anti-thyroid Antibodies
X^m	3.02 ± .6	55th	3/18	7/18	3/18	5/18
X^p	2.67 ± .5	55th	0/7	3/7	0/7	1/7

fertilization. Thus, the question of parental age as a potential indicator of the etiology becomes even more important. Among 45,X live borns there may be a small inverse effect of parental age (Carothers et al. 1980). However, we failed to find a difference *between* the ages of the mothers whose child retained the X^m and the ages of those whose child retained the X^p. These data appear to differ from the report by Hassold et al. (1988) for abortuses in which the mean maternal age of the X^p abortuses (23.8 ± 6.1 years) was younger than the age of the mothers of the X^m abortuses (29.6 ± 5.5 years). Others have also noted an inverse age effect for sex-chromosome monosomy in abortuses (Kajii and Ohama 1979; Warburton et al. 1980) although in their studies the abortuses were not divided on the basis of parental origin of the X. Whether there is (*a*) a separate maternal age–related etiologic mechanism for a subgroup of X-monosomy conceptuses or (*b*) a preferential loss of the X^p-bearing fetus in the younger mother remains to be studied. Nevertheless, the aggregate data from both live-born and aborted 45,X individuals indicate just over a 2:1 ratio of maternal X^m to X^p. This ratio is consistent with the expected proportion of meiotic or mitotic products, with equal loss at each step, given the nonviability of 45,Y cells.

We were unable to detect a relationship between patient phenotype and parental origin of the retained X chromosome. The similar birth weight and subsequent height data argue against a preferentially adverse or favorable fetal environmental effect on growth. While there were a large number of cardiac abnormalities (10), there were no differences between the groups; the number of renal anomalies (three) was too small to assess. Similarly, only three girls had neck webbing, loose nuchal skin, or peripheral edema at birth. While nuchal cystic hygroma is found in a large number of 45,X abortuses (van der Putte 1977), survival appears to be associated with some resolution of the process, and residua are not common when ascertainment is based on karyotype rather than on phenotype. We also looked, retrospectively, at the patient records to see whether any phenotypic finding not prospectively evaluated might distinguish the groups, and none was obvious. If paternal genetic information plays a role in the development and maintenance of placenta and membranes, then either the paternal sex chromosome is not obviously crucial or placental mosaicism exists (Hall 1988, 1990). In the 45,X live born, efforts to detect mosaicism, either in multiple tissues from all germ layers (Burns et al. 1979) or from leukocyte DNA with Y-specific probes (Tho et al. 1990), have been generally unsuccessful. However, placental tissues have not been systematically studied.

The physiologic characteristic we evaluated was the presence of thyroid autoimmunity. We (Schatz et al. 1989) and others (Pai et al. 1977; Germain and Plotnick 1986) have noted a very high prevalence of thyroid autoimmunity in Turner syndrome patients and their families as others have noted in Down syndrome (Fialkow and Uchida 1968). The hypothesis has been that autoimmunity might predispose to nondisjunction, although (*a*) direct evidence for this is lacking and (*b*) a recent report suggests that this is not the case (Torfs et al. 1990). Our data showing a high number but similar proportion of patients with thyroid autoimmunity in both groups suggest that alternate mechanisms should be explored. We considered the hypothesis that the mechanisms responsible for the autoimmunity may, in fact protect the 45,X fetus, allowing it to reach full gestation. While the data of Vadheim et al. (1986) support the hypothesis of selection for fetuses with particular immune constitutions in the case of diabetes mellitus, a recent report (Stagnaro-Green et al. 1990) documents the association of maternal thyroid autoantibodies and pregnancy loss. Further immunologic studies on families in whom there was either a live-born or aborted Turner individual will need to be pursued in order to determine the role/relationship of autoimmunity.

Note added in proof: The recent report by Jacobs et al. (1990), who used molecular techniques in their 45,X live-born population, confirms our data with respect to the just over 2:1 ratio of X^m to X^p chromosomal origin, as well as the lack of a statistical parental age effect and the lack of occult Y mosaicism.

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